conalbumin, ovalbumin, and lysozyme. One of ordinary skill in the art would decide that these proteins cannot be used as a quantitative standard on a gel since their staining intensities do not reflect their quantities.

2. UV absorbance, BCA assay, and Bradford assay (based on Coomassie Blue staining) are commonly used in protein quantity estimation. Many other assays may be used in protein quantitation. One of ordinary skill in the art may use any of these or other assays to estimate protein quantity. Scientists or technicians of the reagent vendor(s) may use any of these or other protein assays to estimate the quantity of conalbumin, ovalbumin, and lysozyme. One of ordinary skill in the art would still decide that these proteins cannot be used as a quantitative standard on a gel since their staining intensities do not reflect their quantities

3. In one case that scientists or technicians of the reagent vendor(s) use only one protein such as BSA as standard. They may still use different assays to estimate protein quantity. In another case that scientists or technicians of the reagent vendor(s) use only one protein assay such as UV absorbance. They may still use different proteins such as BSA, lysozyme, and insulin as standards. The staining intensities still do not reflect protein quantities determined in these cases. One of ordinary skill in the art would still decide that these proteins together cannot be used as a quantitative standard.

4. Many proteins may be used as a protein standard in protein quantitation. Many different assays may be used in protein quantity estimation. Some of the protein quantitation assays may not need a protein standard. For example, UV absorbance can estimate protein quantity by detecting its aromatic amino acid composition without a protein standard. Each protein has different amino acid compositions. Therefore each protein presents different UV absorbance. Since different amino acids have different dye-binding properties, different proteins will have different dye-binding properties. All these facts teach away from the claimed invention that different proteins can be used together in a quantitative protein standard for a protein assay. Using different amounts of BSA to estimate quantities of sample proteins in different assays is commonly used in biotech researches. Combining different amounts of different proteins estimated by BSA to make a protein standard itself is an inventive step. The questions still remain if (1) different detection assays can be used in making the quantitative protein standard (estimating the

amount of each polypeptide in the protein standard), (2) different procedures carried out in solution or on gel can be used in making the quantitative protein standard, and (3) how to make the detection intensities of polypeptides of the quantitative protein standard in using it reflect the amounts estimated in making the standard.

5. Each protein sample contains different buffers, salts, detergents, reducing agents, and impurities. These buffers, salts, detergents, reducing agents, and impurities will affect the detection intensity of a detection assay. When only one protein is used as standard and only one assay is used for quantity estimation, the staining intensities of different proteins will still not reflect their quantities without eliminating the effects of the buffers salts, detergents, reducing agents, and impurities.

6. The claimed invention is made possible only after extensive testing, experimentation, and introduction of inventive steps. It is critical to test if (1) different detection assays can be used in quantity determination, (2) protein quantities determined by one detection assay are reproducible in different experiments, (3) protein quantities determined by one assay are the same in solution and on gel, and (4) staining and destaining time, solution concentration, and temperature variations affect reproducibility. Only after extensive testing, experimentation, and inventive steps are introduced in making and using the quantitative protein standard, the claimed invention is made possible. (1) Different detection assays cannot be used in the protein quantity determination for the polypeptides in one protein standard. Both Coomassie Blue and silver staining are commonly used to estimated protein quantity. If both assays use BSA as standard protein to estimate the quantities of different polypeptides in the quantitative protein standard, the resulting quantitative protein standard can only be used as a size standard. Coomassie Blue, silver staining, or any other detection assays cannot produce detection intensities reflecting protein quantities. (2) Protein quantities determined by one detection assay are reproducible only if same procedure is used in determining quantity of each polypeptide in the quantitative protein standard and in using the quantitative protein standard. If Coomassie Blue staining is used in using the quantitative protein standard on a gel, the quantitative protein standard has to be made with the Coomassie Blue staining on a gel and vice versa. (3) Protein quantities determined by one assay with same protein such as BSA as a standard are not same in solution and on a gel. Coomassie

Blue staining is commonly used both in solution and on gel protein staining. From extensive experiments, the applicant reasoned that gel electrophoresis, staining and destaining procedures eliminate the effects of different buffers, salts, detergents, reducing agents and impurities in different protein samples. Therefore on gel staining results are more accurate than in solution staining in protein amount estimation. This is why Coomassie Blue staining on a gel is used in protein quantity estimation in making the quantitative protein standard for each of the polypeptides in all examples of the disclosed invention. This invention may have general applications on protein quantity estimation. For example, chromatography on a column may separate different buffers, salts, detergents, reducing agents and impurities. Detecting a protein quantity after gel electrophoresis or column chromatography by a detection assay such as UV absorbance will be more accurate than that without electrophoresis or chromatography procedures. (4) Staining and destaining variations such as time, solution concentration, and temperature affect reproducibility and the procedures recommended in the disclosed Examples should be used to obtain reproducible results. All these are inventive steps in the claimed invention. Involvement of extensive testing, experiments and introduction of multiple inventive steps in making and using the quantitative protein standard indicate it is not obvious to one of ordinary skill in the art at the time of the instant invention to make the quantitative protein standard as taught by the instant invention.

7. The claimed quantitative protein standard, the methods of making and using the standard are integral parts of the claimed invention. When the claimed method of using the protein standard is used in estimating the amount of a sample protein, only the claimed quantitative protein standard can be used to assure accurate protein quantity estimation. When protein standards other than the claimed quantitative protein standard are used, the staining intensities will not reflect the protein amounts. Since the method of using the quantitative protein is not obvious, the claimed quantitative protein standard and method of making are not obvious to one of ordinary skill in the art either. As a matter of fact, the claimed quantitative protein standard, the method of making and using the standard are invented simultaneously. Without the claimed quantitative protein standard and method of making it, the method of using the standard would not have been

developed. New claim 56 is added to reflect the integrity of methods of making and using the claimed quantitative protein standard.

8. When different amounts of BSA are used as protein standards on a gel to estimate quantities of each polypeptide of the quantitative protein standard, the staining intensities of the polypeptides still do not reflect the amount of the polypeptides if different staining assays are used in making the quantitative protein standard or in using it. There are tens of staining assays available for staining a gel after electrophoresis. These assays include Coomassie Blue (Groth et al, Biochem. Biophys. Acta 71, 377, 1963), Remazol Blue (Datyner et al, Anal. Biochem., 52: 45-55, 1973), Amido Black (Wilson, Anal. Biochem., 96: 263-278, 1979), Fast Green (Wilson, Anal. Biochem., 96: 263-278, 1979), Fluorescamine (Ragland et al, Anal. Biochem., 59: 24-33, 1974), Carbocyanine (Green et al, Anal. Biochem., 56: 43-51), Sodium Acetate (Nelles et al, Anal. Biochem., 73: 522-531, 1976), Silver (Ocbs et al., Electrophoresis, 2: 304-307, 1981), Copper and Zinc (BioRad Laboratories, Life Science Research Product Catalog, page 116, 1988) stains to name a few. Each of these stains has different mechanism of protein staining. Only one staining assay can be used in detecting and estimating quantity of each of the polypeptides of the quantitative protein standard. In addition, the same staining assay has to be used in using the quantitative protein standard. These are the inventive steps of the claimed invention. Without these and other inventive steps, the claimed invention will not be possible. These inventive steps also demonstrate that the methods of making and using the claimed quantitative protein standard are integral parts of the claimed invention.

9. The invention solves a long-felt, long-existing, but unsolved need. The applicant has been working on protein sizing and quantification for over 20 years. It is always painful for the applicant and other researchers to use the laborious method taught by Fishbein (similar as that taught by Houghton et al, US patent no. 6,168,946). This laborious and costly method is still used today in many academic and industrial labs as revealed recently by Houghton et al. Therefore solution of the long-felt and unsolved need further indicates the application is not obvious.

10. The patent of Mizutani was granted on April 29, 1975 which is over 30 years ago. Coomassie Blue used for protein staining after electrophoresis was published over 40 years ago (Groth et al, Biochem. Biophys. Acta 71, 377, 1963). The method for

estimating the amount of sample protein using different amount of a standard protein on a gel has been available since the publication of Fishbein (Anal. Biochem., 46, 388-401, 1972) which was over 30 years ago. BSA is one of the most commonly used proteins. It has been used as a protein standard in protein amount estimation ever since these detection assays were published. Because of obvious advantages of saving time, labor, and cost with the claimed invention, those skilled in the art surely would have implemented it by now. The fact of lack of implementation for over 30 years indicates the claimed invention is not obvious.

In conclusion, the disclosed invention involves extensive testing, experimentation, and introduction of inventive steps. It is not obvious over the proteins taught by Mizutani, over using BSA as a standard in protein amount estimation, or the combination of them. Therefore the amended and new claims are submitted that patentable subject matter is clearly presented. If the examiner agrees but does not feel that the present claims are technically adequate, applicant respectfully requests that the examiner write acceptable claims pursuant to MPEP 707.07(j).